SWELLING OF AGAR IN SOLUTIONS OF AMINO ACIDS AND RELATED COMPOUNDS

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(WITH SIX FIGURES)

A study of the behavior of a large variety of substances in aqueous solution on the hydration of agar has shown that there are very few such solutions in which agar swells to a greater degree than it does in distilled water. There are some substances, however, which increase the hydration of agar above that attained in pure water. These are the amino acids. The amino compounds are of such immediate biological importance that a discussion of their action deserves special consideration, and may aid in explaining the scattered results obtained by various workers in which increased total growth and apparently catalytically accelerated actions have been obtained by the addition of certain amino acids to culture solutions.

The purification of the agar, as well as the preparation of the agar plates and the instruments and methods for measuring the swelling, have already been described in detail by MacDougal.[1] The results here discussed were obtained by the application of these methods. The amount of swelling in water is taken as the standard. Thus the percentage of swelling of the dried plates in water is expressed as 100, and that in the various solutions is calculated on this basis.

Swelling of agar in solutions of amino acids

The dried agar plate, prepared from the specially purified agar and used in this experiment, showed a total swelling in distilled water of 2000 per cent. The amino acids, glycocoll, alanin, and phenylalanin were used in 0.01 normal concentration. The solutions in which the agar plates were allowed to swell were renewed every 24 hours; the swellings were complete after about 6 days. The results are given in table I.

[1] MacDougal, D. T., Auxographic measurement of swelling of biocolloids and of plants. Bot. Gaz. 70: 126–136. 1920.

In table II are given the results of some earlier experiments made with plates prepared from the "bacto-agar" of the Digestive Ferments Company. The swellings in the solutions of the corre-

TABLE I

SWELLING OF AGAR PLATES 0.11 MM. IN THICKNESS AT 15°C. IN 0.01 NORMAL SOLUTIONS OF AMINO ACIDS, CALCULATED ON BASIS OF SWELLING IN WATER TAKEN AS 100; SOLUTIONS RENEWED EVERY 24 HOURS; SWELLING OF AGAR PLATES IN WATER 2000 PER CENT

Water	Glycocoll	Alanin	Phenylalanin
100..............	165	151	161

sponding organic acids are also given for comparison. Thus it can be seen that in acetic acid agar exhibits a much lower swelling than in the a-amino compound, glycocoll. The same relation is maintained with the other acids.

TABLE II

SWELLING OF AGAR PLATES 0.10–0.23 MM. IN THICKNESS AT 16–17°C. IN SOLUTIONS OF AMINO ACIDS AND CORRESPONDING ORGANIC ACIDS, CALCULATED ON BASIS OF SWELLING IN WATER TAKEN AS 100; DRIED PLATES SWELLED 2570 PER CENT IN WATER; DURATION OF SWELLING 20–60 HOURS, DURING WHICH TIME SOLUTIONS WERE NOT RENEWED

Normal concentration	Water	Glycocoll	Acetic acid	Alanin	Propionic acid	Asparagine	Aspartic acid	Succinic acid
0.01...........	100	115	61	94	51	91	49	49
0.002..........	100	123	59	108	63	94	58	62
0.0004.........	100	103	76	....	70	106	69	68
0.00008........	100	....	102	....	....	126	81	98

It is worthy of note that agar behaves quite differently from gelatine in relation to acids and bases, and that this also applies to the amino acids, as shown in table III. Agar is a carbohydrate, and as such exhibits some of the properties of an exceedingly weak acid. DR. MCGEE of this laboratory determined by means of the indicator method[2] that in 0.75 per cent solution the purified agar shows a hydrogen ion concentration expressed by $P_H = 6.5$.

[2] DUGGAR, B. M., The use of the colorimeter in the indicator method of H ion determination with biological fluids. Ann. Mo. Bot. Gard. **6**:61–70. 1918.

———, The micro-colorimeter in the indicator method of hydrogen ion determination. *Ibid.*, **6**:179–181. 1919.

It has long been known that carbohydrates form salts with metals, and that they react with the chlorides, sulphates, and other salts of the heavy metals, such as copper, lead, mercury, gold, iron, silver, etc., to form the corresponding carbohydrate salt with

TABLE III

SWELLING OF DRIED GELATINE PLATES 0.5 MM. IN THICKNESS AT 16–17° C.; SWELLING IN WATER 600 PER CENT

Normal concentration	Water	Propionic acid	Alanin
0.1	100	....	91
0.05	100	256	83
0.01	100	185	88
0.002	100	130	86
0.004	100	83	75

the liberation of hydrochloric, sulphuric, or other acids. McGEE has determined the hydrogen ion concentration of a number of heavy metal salts in 2 per cent aqueous solution, and also in mixtures of 2 per cent of the salts plus 2 per cent d-glucose. A 2 per cent solution of d-glucose in water showed $P_H = 6.6$.

TABLE IV

HYDROGEN ION CONCENTRATION EXPRESSED AS P_H OF 2 PER CENT SOLUTIONS OF SOME HEAVY METAL SALTS AND OF SAME MIXED WITH 2 PER CENT D-GLUCOSE

$ZnCl_2$	6.3	$AgNO_3$	5.5	$HgCl_2$	3.9	$CuSO_4$	4.6
$ZnCl_2$ + glucose ...	5.4	$AgNO_3$ + glucose...	5.3	$HgCl_2$ + glucose...	3.8	$CuSO_4$ + glucose...	4.4

It is apparent that in the addition of the glucose to the heavy metal salts the acidity of the mixture is appreciably raised.

The amino acids being amphoteric electrolytes, it is to be expected that they would behave like acids toward bases, and like bases toward acids. Furthermore, there are a number of reactions of which the amino acids are capable which may be of importance in interpreting their behavior toward agar. Thus the simplest amino acid, glycocoll (NH_2CH_2COOH), can apparently give rise to an internal salt, NH_3CH_2COO. Glycocoll in solution would then exist in equilibrium as the un-ionized

NH_2CH_2COOH together with its ions and with NH_3CH_2COO, as well as a hydrated form $OHNH_3CH_2COOH$. The existence of this latter compound has been used to explain why acids such as glycocoll do not follow the simple Ostwald dilution law. The recent observations of BIRCKNER[3] on the interaction of ethyl alcohol and certain amino acids may be interpreted in favor of the theory of salt formation of the alcohol with the amino group. Further insight as to whether the increased swelling of agar in amino acids is due to simple salt formation or to the formation of a compound related to the form $OHNH_3CH_2COOH$ was sought in a study of the behavior of agar toward ammonium hydroxide and some related substances.

Swelling of agar in alkaline hydroxides and in ammonium salts

A study of the behavior of agar in solutions of various alkaline hydroxides revealed a number of facts worthy of notice. The first experiments were conducted in the usual manner with 25 cc. of the hydroxide solutions in the dishes containing the pieces of agar. An examination of the auxograph record of the swelling thus obtained showed a remarkable similarity, in that after about 24 hours there was a marked acceleration in the rate of swelling, and the final results were very similar in all cases. In figs. 1 and 2 the curves of swelling are reproduced for potassium hydroxide and ammonium hydroxide. The total swellings thus obtained in the various solutions are given in table V.

TABLE V

SWELLING OF DRIED PLATES OF AGAR IN 25 CC. OF ALKALINE HYDROXIDE SOLUTIONS AT 15°C.; TOTAL SWELLING OF AGAR PLATES IN WATER 3950 PER CENT

Normal concentrations	Water	NH_4OH	LiOH	NaOH	KOH
0.01	100	63	60	55	49
0.001	100	77	77	75	81

Titrations after 24 hours of the solutions in which the agar had been swelling showed that the solutions had decreased

[3] BIRCKNER, V., Jour. Biochem. 38:245–254. 1919.

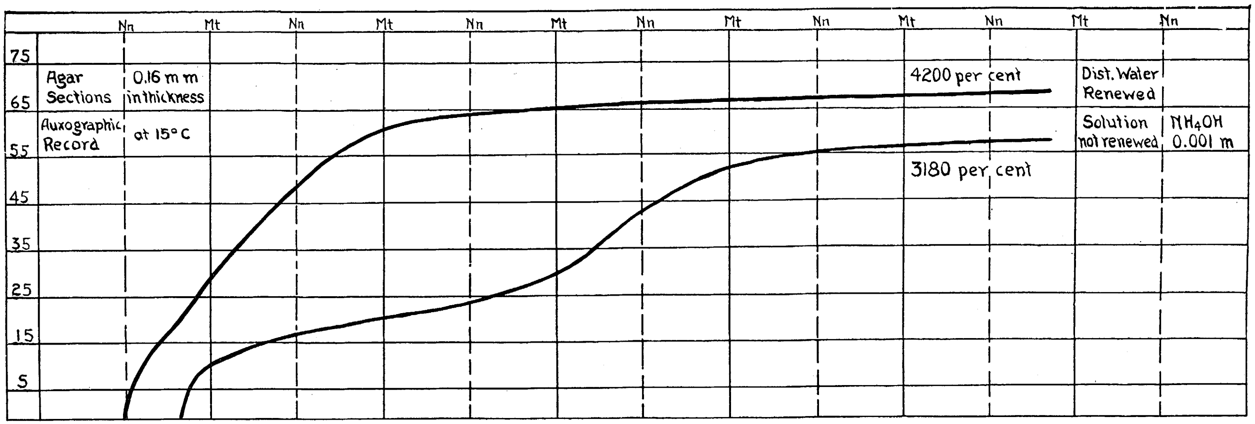

FIG. 1.—Auxographic record of swelling of agar plates in 0.001N solution of NH_4OH not renewed, and in water renewed every 24 hours

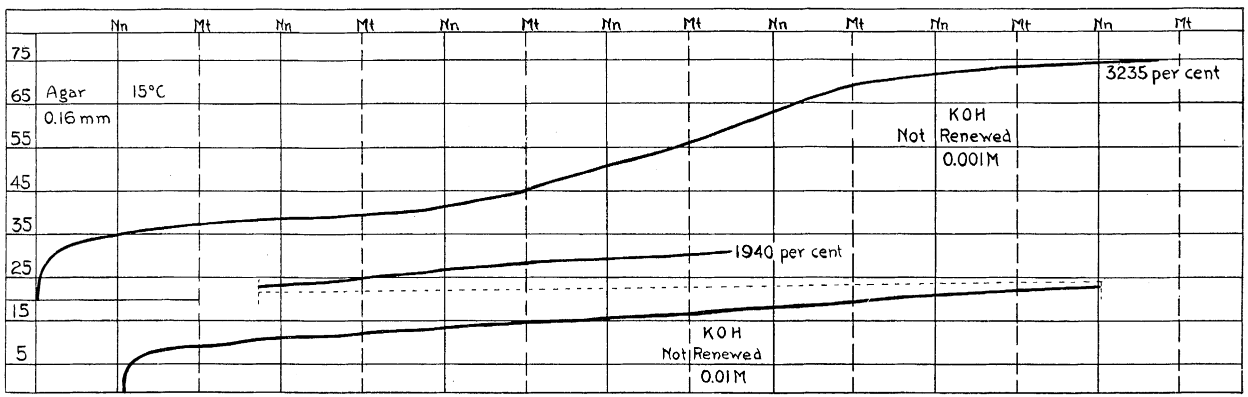

FIG. 2.—Auxographic records of swelling of agar plates in 0.01 and 0.001N solutions of KOH not renewed during course of experiment.

considerably in strength in this time, probably due to absorption of CO_2 from the air, and, in the case of the ammonium hydroxide, to volatilization. Experiments were then made in which the solutions were removed from the agar and replaced by fresh solutions every 12 or 24 hours. The results thus obtained differ radically from the previous ones, as to form of the record made by the swelling agar, as well as to the total amount of swelling attained in each case. Figs. 3 and 4 give the auxograph record of the swelling agar in solutions renewed every 12 hours. The relatively sudden increase in rate of swelling after 24 hours, which was so striking in the experiments in which the solutions were not renewed, is entirely absent. This accelerated swelling undoubtedly represents the rate of swelling in a solution which is but slightly alkaline and approaches that obtained in water. Furthermore, the total swelling of agar in KOH, NaOH, and LiOH is decidedly lower in the solutions which had been renewed. Especially noteworthy, however, is the fact that ammonium hydroxide in 0.001 normal concentration produces a swelling considerably in excess of water when the solution is renewed. This observation has been verified repeatedly.

The differences in the swelling of agar in NH_4OH and $C_2H_5NH_2$ on the one hand, and in LiOH and KOH on the other hand, are considerable, particularly in the more dilute solutions.

TABLE VI

SWELLING OF DRIED AGAR PLATES AT 15°C. IN ALKALINE HYDROXIDE SOLUTIONS, RENEWED EVERY 12 HOURS; TOTAL SWELLING OF DRIED AGAR PLATE IN WATER 3950 PER CENT

Normal concentration	Water	NH_4OH	Ethyl amine	LiOH	NaOH	KOH
0.01	100	25	31	24	21	21
0.001	100	115	88	40	35	29

Owing to the fact that in solutions of ammonium hydroxide and ethyl amine there exist equilibria respectively between dissolved NH_3 and the hydroxide, and between dissolved $C_2H_5NH_2$ and its hydroxide, the condition in solutions of these substances, particularly in the more concentrated solutions, offers a rather complicated situation not dissimilar from that obtaining in solutions

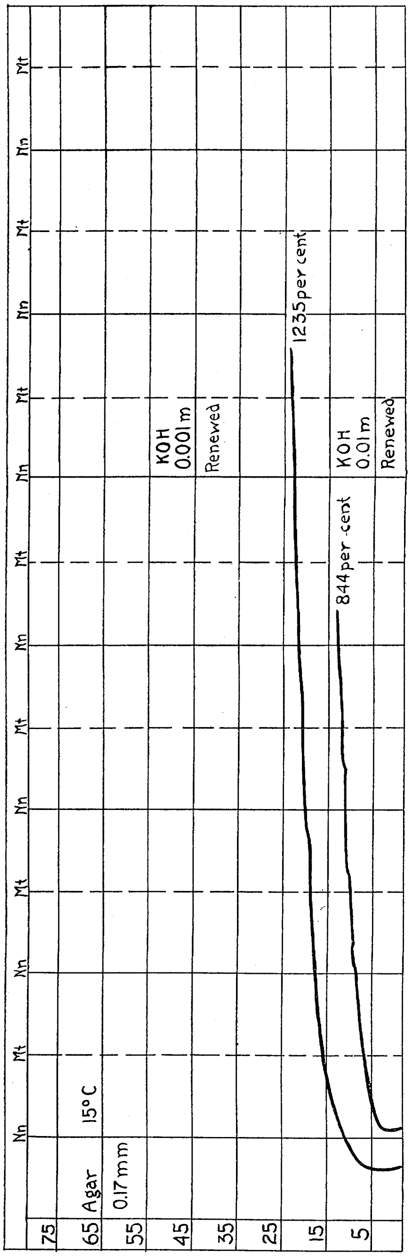

FIG. 3.—Auxographic records of swelling of agar plates in 0.01 and 0.001N solutions of KOH renewed every 12 hours

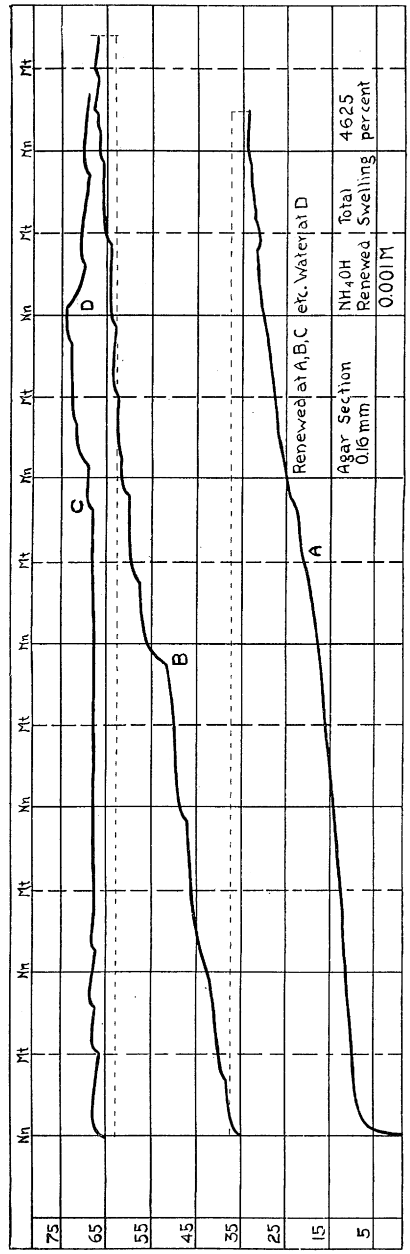

FIG. 4.—Auxographic record of swelling of agar plates in 0.001N solution of NH_4OH renewed frequently and replaced by water at *D*

of the amino acids. From these experiments it would appear that the stronger the base, as indicated by its position in the electromotive series, the less is the effect on the swelling of agar. Thus we have in effect K<Na<Li, and, in the weaker concentrations, ethyl amine, which is a stronger base than ammonium hydroxide, falling below this in hydration capacity. Interesting is the case of aniline, a very weak base. Separate experiments with this substance were carried out, in which agar showed the following swelling: in 0.01N solution, 110; in 0.001N solution, 100; in water, 100.

Further investigation of the effect of a possible agar-salt formation on the swelling was undertaken by a study of the behavior of dried agar plate which had been prepared from agar previously treated with KOH and NH_4OH. Portions of the purified agar were allowed to remain in such a quantity of 0.01 normal KOH and NH_4OH solutions as to make up a 2.5 per cent agar solution. After about 14 hours these solutions with the agar were heated, and the agar plates poured and then dried in the usual manner. The "kaliated" and "ammonated" plates thus prepared were then allowed to swell in solutions of KOH, NH_4OH, and distilled water. During the first 24 hours the water in which these plates had swelled became distinctly alkaline. Thereafter the water was renewed every 48 hours and showed but slight alkaline reaction. Thus the hydrogen ion concentration of the water in which the plates had been swelled, as determined by the indicator method, gave the values shown in table VII.

TABLE VII

Time	"Kaliated" plate P_H	"Ammonated" plate P_H
August 13	10.0	10.0
August 15	7.4	7.2
August 17	7.7	7.4

The figures given for August 15 and 17 probably indicate the values representing the products of hydrolysis of the "kaliated" and "ammonated" plates. The total swellings of these plates are given in table VIII.

From the results thus far obtained it would appear that the swelling of agar in water is surpassed only by that taking place

under conditions favorable to the formation of a combination of agar with ammonia or its closely related compounds. The exact nature of these agar combinations and the manner in which water acts upon them have not as yet been determined with any degree

TABLE VIII

SWELLING OF DRIED "KALIATED" AND "AMMONATED" AGAR PLATES AT 15°C.; ACTUAL SWELLING IN WATER OF "KALIATED" PLATE 3450 PER CENT; "AMMONATED" PLATE 2450 PER CENT

Plate	Water	0.01 N KOH	0.001 N KOH	0.01 N NH_4OH	0.001 N NH_4OH
"Kaliated"	100	57	83	....	....
"Ammonated"	100	....	....	107	122

of satisfaction. Ammonium salts, such as ammonium chloride and ammonium acetate, in the concentrations thus far tried, have shown no augmenting effect on the swelling. Ammonium acetate, although neutral in water solution, is hydrolyzed to a considerable extent with the formation of the ions of ammonium and acetic acid. The effect of these salts, in which the nitrogen can be considered as fully satisfied, on the swelling of agar shows a typical neutral salt effect.

TABLE IX

SWELLING OF DRIED PLATES OF AGAR AT 15°C. IN SOLUTIONS OF AMMONIUM CHLORIDE AND AMMONIUM ACETATE; TOTAL SWELLING OF AGAR PLATES IN WATER 1700 PER CENT

Normal concentration	Water	NH_4Cl	$NH_4CO_2CH_3$
0.01	100	58	58
0.001	100	94	100

An examination of the records produced by swelling agar in which the solutions are regularly renewed indicates clearly that the addition of a fresh solution of ammonium hydroxide affects distinctly the subsequent swelling of the already partially hydrated agar (figs. 3, 4). Thus, for example, sections of agar had swelled to values in water = 100; in 0.01N NH_4OH = 25. After the sections had attained their full swelling of 25 in 0.01N NH_4OH, the hydroxide solution was replaced by water. The agar swelled further to a value equal to 73. Thereupon other sections of dried

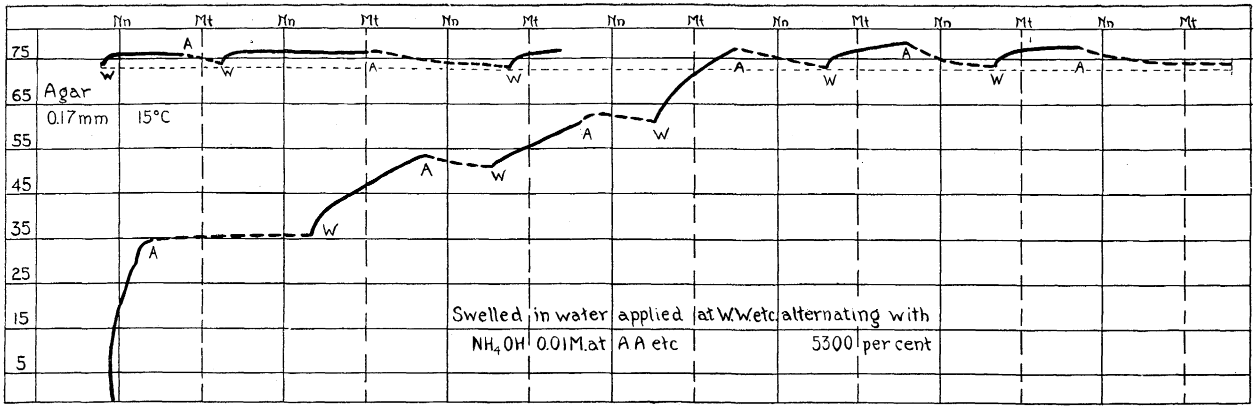

FIG. 5.—Auxographic record of swelling of agar plates in 0.01N solution of NH_4OH alternated every 24 hours with water; broken line indicating swelling in NH_4OH.

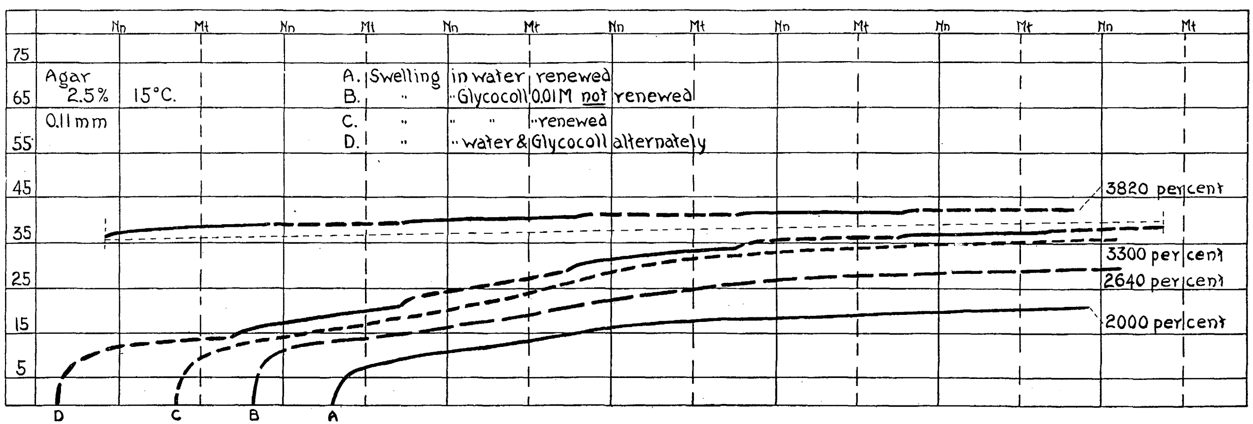

FIG. 6.—Auxographic records of swelling of agar plates: *A*, in water renewed every 12 hours; *B*, in 0.01N solution of glycocoll not renewed; *C*, in 0.01N solution of glycocoll renewed every 24 hours; *D*, water and 0.01N solution of glycocoll alternating; broken line indicating swelling in glycocoll.

agar plate were allowed to swell by alternating every 12 hours water and ammonium hydroxide in 0.01N solution. The curve for this swelling is reproduced in fig. 5. At first both the NH_4OH and the water cause swelling. As the hydration continues, however, the swelling in NH_4OH becomes slight, and finally there is an actual shrinkage in the hydroxide with, moreover, a subsequent swelling again in the water. The total swelling thus attained greatly exceeds that in water or ammonium hydroxide alone. The total swelling of the dried agar plates treated thus alternately with water and ammonium hydroxide was 5300 per cent, or on the basis of the swelling in water equal to 100, the swelling in alternating NH_4OH and H_2O equaled 134. Similar experiments were carried out in which glycocoll was alternated with water. Thus with 18 changes in 15 days agar swelled 191 as against 100 in water. In a solution of glycocoll which was not changed during the entire swelling the agar showed a swelling value of 132. The curves thus formed by the swelling of agar in glycocoll are shown in fig. 6.

No adequate explanation has been found for the augmenting effect on the swelling of agar produced by ammonia and the amino acids and here described. From a consideration of the relative effects of various hydroxides it would appear that something analogous to salt formation and subsequent hydrolysis of this compound may be involved.[4] Ammonium, as a weak base united with agar, an exceedingly weak acid, would form a salt which would very easily be hydrolyzed. Under conditions in which this hydrolysis is suppressed by the presence of a common ion (NH_4), the excessive swelling does not take place. Thus when dried agar plates are allowed to swell by alternating solutions of 0.001N, NH_4OH, and NH_4Cl the total swelling does not exceed that attained in water, but actually falls somewhat below that value.

The possible biological significance of these changes in volume, which are also exhibited by agar-protein mixtures with respect to growth and metabolism, seems to be so great as to warrant, not only this brief description, but also the formation of plans for the extension of the experiments.

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[4] BRACEWELL, R. S., Jour. Amer. Chem. Soc. **41**:1511–1515. 1919.